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Multivariate analysis of elution parameters for RP-HPLC with charged aerosol detection of sucrose monoester regioisomers



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INTRODUCTION

Sugar fatty acid monoesters have been shown to possess antibiotic properties, and enzyme-catalysed regioselective synthesis of these compounds is a very efficient alternative to conventional organic synthesis. The physical and chemical properties of sugar fatty acid esters depend on the saccharide moiety, fatty acid chain length, and position and degree of esterification. Sucrose esterification with a single fatty acid potentially results in a total of 255 isomers, eight of which are monoester regioisomers.

In the present work, the use of step-down gradient elution profiles to improve separation of sucrose caprate regioisomers [1] was investigated as part of the development of a quantitative RP-HPLC analysis method with charged aerosol detection (CAD). The investigation was conducted using design-of-experiments methodology and evaluated by multivariate regression analysis [2].

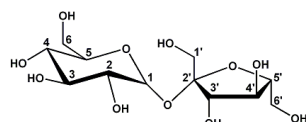


Figure 1: Sucrose structure.
Haworth perspective formula of sucrose with carbon atom numbering.

DESIGN OF EXPERIMENTS AND MULTIVARIATE ANALYSIS

The HPLC analysis of sucrose caprate was investigated using a design-of-experiments approach, with the gradient elution profiles described by four variables. The performed design was a face-centred composite (FCC) optimization design, giving a 4-cube design space, with each variable investigated at three levels. The resulting regioisomer retention times were modelled using partial least-squares (PLS) regression, and the model was iteratively optimized through elimination of insignificant variable effects and recalculation.

The regression analysis showed the four elution variables to have effects of varying significance. The concentration variables (A and C) had the most significant effects on the retention times, both as individual terms and as part of variable interactions. The time variables (B and D) were only significant as part of interaction effects involving one or both of the concentration variables.

Table 1: Design variables. Variable symbols, descriptions and ranges.

Variable	Description	Range
A (%)	Initial acetonitrile concentration	30-40
B (min)	Duration of initial concentration	2-6
C (%)	Mid-section acetonitrile concentration	20-40
D (min)	Duration of mid-section concentration	1-5

RP-HPLC SEPARATION OF SUCROSE CAPRATE REGIOISOMERS

RP-HPLC analysis of sucrose caprate, with eluent consisting of acetonitrile and water and C18-column, resulted in the resolution of the eight possible regioisomers with $R_s \geq 1.3$. All regioisomer structures were elucidated by NMR-spectroscopy, and CAD exhibited detection of sucrose caprate with a sensitivity of 10-100 ng.

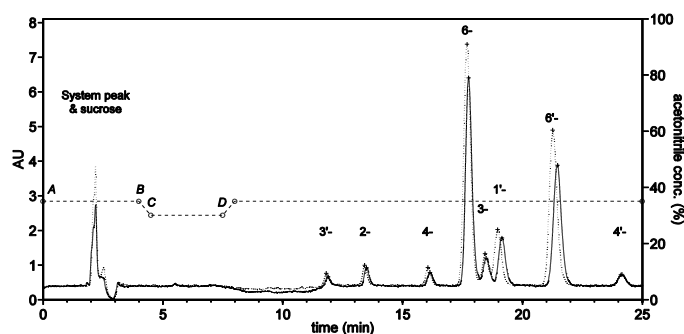


Figure 2: Chromatograms from RP-HPLC analysis of sucrose caprate, with profile of eluent acetonitrile concentration (---).

Elutions with TFA (—) and without TFA (---) added to the eluents are shown. Variables A, B, C and D describe the elution profile: A = 35 %, B = 4 min, C = 30 %, D = 3 min. R_s -values: 3'-, N/A; 2-, 4.8; 4-, 6.8; 3-, 3.6; 3-, 1.36; 1-, 1.3; 6-, 4.1; 4-, 4.3.

ISOCRATIC ELUTIONS OF SUCROSE ALKANOATES

Isocratic elutions of sucrose caprate and sucrose laurate showed that increasing the eluent acetonitrile concentration decreased retention time for all the sucrose alkanate regioisomers. Three regioisomers, 6, 3 and 1', showed similar elution properties for each of the sucrose alkanates. The 3-regioisomers were resolved below 38 % and 41 % acetonitrile, respectively, while above the indicated concentrations they co-eluted with their respectively succeeding 1'-regioisomers.

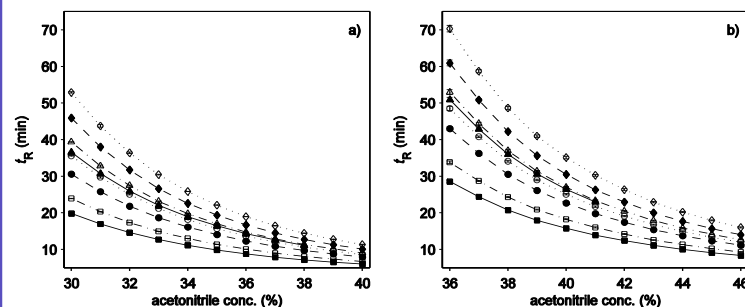


Figure 3: Regioisomer retention time as function of eluent acetonitrile concentration for isocratic elutions of sucrose caprate (a) and sucrose laurate (b). Regioisomers are arranged from bottom to top according to elution order: —■ 3', —□ 2, —● 4, —○ 6, —▲ 3, —△ 1', —◆ 6', —◇ 4'. Error bars indicate standard deviations.

EVALUATION OF ELUTION EFFICIENCY

Resolutions, expressed as R_s -values, were calculated from retention times and peak widths. The two R_s -values for the separation of sucrose caprate regioisomers 6, 3 and 1' were evaluated using an aggregate objective function (AOF) denominated resolution deviation (RD) and defined as

$$RD(R_s) = a \times \sqrt{\frac{1}{2} \sum_{i=1}^2 \min(R_{si} - 1.5, 0)^2}$$

where $a = 1/1.5$ is a scaling factor for an output range of [0, 1], with 0 representing optimal resolution.

Isocratic elution with 34 % acetonitrile concentration resulted in lowest RD, while higher acetonitrile concentrations resulted in decreased analysis time but increased RD. Gradient elution resulted in RD in the same range, while analysis time was further reduced. For the RD-range 0.15-0.16, analysis time was reduced by 28 %, and for the RD-range 0.095-0.11 the average analysis time reduction obtained was 7 %.

Table 2: Elution efficiency.

a) Isocratic elutions			b) Gradient elutions		
Acetonitrile conc. (%)	Resolution deviation	Analysis time (min)	A (%), B (min), C (%), D (min)	Resolution deviation	Analysis time (min)
31	0.24 (3.8)	47	35, 4, 30, 3	0.11 (2.0)	24
32	0.25 (4.4)	39	35, 6, 30, 1	0.15 (2.0)	23
33	0.16 (3.9)	32	35, 2, 30, 1	0.15 (2.0)	23
34	0.096 (73)	27	30, 4, 30, 1	0.095 (20)	26
35	0.20 (1.7)	23			
36	0.23 (1.4)	20			
37	0.35 (2.5)	17			

CONCLUSION

Methods for RP-HPLC analysis of sucrose alkanate regioisomers were developed where resolutions above the level of quantification adequacy, $R_s \geq 1.0$, were achieved for all regioisomers.

Isocratic elution of sucrose caprate with 34 % acetonitrile resulted in the best separation, while gradient elution resulted in similar RD, but reduced the analysis time by 7-28 %.

All the regioisomers of sucrose caprate were successfully identified and assigned, and charged aerosol detection provided a mass-sensitivity of 10-100 ng.

Design of experiments and multivariate analysis were shown to be applicable and useful tools for method development in RP-HPLC analysis of sucrose fatty acid esters.

Resolution deviation (RD) was defined as an aggregate objective function for evaluating the separation of the sucrose caprate regioisomers.

REFERENCES

1. Ritthitham S, Wimmer R, Stensballe A, Pedersen LH. (2009) J. Chromatogr. A, 1216: 4963
2. Lie A, Wimmer R, Pedersen LH. (2013) J. Chromatogr. A, 1281: 67